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Assessment of Use of the COBAS AMPLICOR System with BACTEC 12B Cultures for Rapid Detection of Frequently Identified Mycobacteria

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The use of the COBAS AMPLICOR System (Roche Molecular Diagnostics, Basel, Switzerland), the only automated system for PCR testing, was evaluated for a rapid identification of mycobacteria with positive BACTEC 12B cultures. Two hundred ninety-six specimens with a growth index of ≥30 were analyzed for the presence of *Mycobacterium tuberculosis* complex, *Mycobacterium avium*, and *Mycobacterium intracellulare*. Compared to traditional methods and provided that samples with PCR inhibition are retested at a 1:10 dilution, the sensitivity and specificity of the COBAS AMPLICOR System with BACTEC 12B cultures were 100 and 98%, respectively. The COBAS AMPLICOR method is rapid and reliable for identifying the most common mycobacteria in cultures.

The traditional detection of mycobacteria is based on microscopic examination of the specimens stained with Ziehl-Neelsen stain or fluorescent dye and specific culture techniques. Over the last 10 years, cultures on liquid medium (BACTEC 12B; Becton Dickinson) coupled with probe hybridization have contributed to a more rapid detection and identification of mycobacteria than solid cultures and conventional identification (11, 15). Recently, molecular amplification methods, such as PCR, have increased the sensitivity of assays for the direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens (3, 4, 7, 8). Many evaluations of the commercial PCR test (Amplicor MTB assay; Roche Diagnostic Systems, Rotkreuz, Switzerland) displayed a high specificity but a variable sensitivity, which was near 95% for smear-positive specimens but only 60% for smear-negative specimens (1, 9). An automated system was developed (COBAS AMPLICOR MTB system; Roche Diagnostic Systems) for the amplification and detection process. The first published evaluations from experiments with direct specimens (2, 17) were disappointing because the sensitivity of the automated system was not better than that of the manual system. The purpose of the present study was to evaluate the rapidity and reliability of the automated commercial COBAS AMPLICOR system on BACTEC 12B broth cultures with a growth index (GI) of ≥30, for the identification of *M. tuberculosis* complex, *Mycobacterium avium*, or *Mycobacterium intracellulare*. The results were compared with the identification by commercial probe hybridization (Accuprobe; Gen-Probe Inc., San Diego, Calif.) and biochemical identification of mycobacteria.

Specimens (n = 2,268) of various origins (respiratory and nonrespiratory specimens, with the exception of blood cultures) were screened by microscopy for acid-fast bacilli (AFB) and cultured on BACTEC 12B, Coletsos, and Stonebrink media. From all BACTEC 12B cultures with a GI of ≥30 (n = 296), 300 μl of liquid medium was taken and stored at −20°C. A run with 100 μl of each stock sample was processed weekly according to the protocol provided by the manufacturer for the COBAS AMPLICOR test intended for direct amplification of respiratory specimens (12). The amplification products were tested with three probes specific for *M. tuberculosis* complex, *M. avium*, and *M. intracellulare*. An internal control (Myco IC) recently described by Rosenstraus et al. (13) was performed in all specimens to monitor the possible inhibition of amplification. The cutoff for a positive value was an optical density (OD) of 0.350. Inhibited specimens (Myco IC OD, <0.350) were retested after a 1:10 and 1:100 dilution when the 1:10 dilution remained inhibited. The PCR results were compared with those obtained with Accuprobe hybridization applied to BACTEC cultures at GIs of ≥999 or with the identification from subcultures on solid media. All specimens with discordant results were retested with the PCR procedure.

Of the 296 specimens with a GI of ≥30 in BACTEC 12B cultures, 125 were culture positive for mycobacteria (42%), 88 (30%) were contaminated by microorganisms other than mycobacteria, and 83 (28%) were negative on subcultures (Table 1). The sensitivity of the COBAS AMPLICOR system with BACTEC 12B cultures was 100% and the specificity was 98%. No positive PCR results with BACTEC cultures positive for mycobacterial species other than *M. tuberculosis* complex, *M. avium*, or *M. intracellulare* were obtained. No differences between the groups of respiratory or nonrespiratory specimens for the detection and identification of mycobacteria were observed. The rate of amplification inhibition was high (57 of 296 specimens [19%]), without a predominance of any type of sample. When positive BACTEC cultures were diluted 1:10, 51 of 57 lost the inhibitors. A 1:100 dilution removed the inhibition in the six remaining specimens.

Three samples showed divergent results by PCR and conventional identification methods. Two samples were considered to have false-positive PCR results for *M. tuberculosis* complex, a joint fluid (BACTEC 12B; GI = 118), and a pleural effusion (GI = 82). For these two specimens, the PCR OD values were 0.641 and 1.233, respectively, for the first assays and 0.467 and 0.018, respectively, for the second assays. The interval between the first and second PCR assay was about 2 months. The two patients presented no clinical evidence of tuberculosis. For the first patient, six other joint fluids were

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TABLE 1. Comparison of COBAS AMPLICOR PCR and culture results for the detection of mycobacteria in 296 prospective BACTEC 12B cultures with a GI of ≥30

PCR result	No. of BACTEC 12B cultures (GI, ≥30)					
	Positive for mycobacteria				Contaminated	Negative
	Mtb[a]	Mav[b]	Mint[c]	Other species		
Positive for Mtb[a]	108	0	0	0	1	2
Positive for Mav[b]	0	8	0	0	0	0
Positive for Mint[c]	0	0	2	0	0	0
Negative	0	0	0	7	87	81

[a] Mtb, *M. tuberculosis* complex.
[b] Mav, *M. avium*.
[c] Mint, *M. intracellulare*.

culture negative for the mycobacteria. For the second patient, no other specimen was cultured and the clinical presentation was not compatible with tuberculosis. The third specimen (abdominal ganglion; GI = 999) had a positive PCR result for *M. tuberculosis* complex, but the cultures were contaminated by bacteria other than mycobacteria. The PCR OD value from the first assay was 0.661, and the value from the second assay was 0.021. This sample most probably contained mycobacteria since AFB were microscopically detected. The patient presented abdominal pain but was not compliant and refused antimycobacterial treatment.

The time required to detect mycobacteria in BACTEC 12B cultures with the COBAS AMPLICOR system (BACTEC GI, ≥30) was compared with the detection by Ziehl-Neelsen staining (BACTEC GI, ≥100) and Accuprobe identification (BACTEC GI, ≥999). The time savings for the identification of mycobacteria with the COBAS AMPLICOR system were marginal (0.5 to 1.5 days) compared to that of Ziehl-Neelsen staining of BACTEC 12B cultures but were almost 1 week compared to that of Accuprobe hybridization. If the COBAS AMPLICOR system were used only at a GI of ≥100 when Ziehl-Neelsen staining was positive for mycobacteria, the time savings would be 4.5 days for *M. tuberculosis* complex, 3.5 days for *M. avium*, and 3 days for *M. intracellulare*.

Our findings confirm the value of the Roche PCR assay for the rapid detection and identification of mycobacteria in BACTEC 12B broth cultures. The COBAS AMPLICOR system allowed us to identify 118 (93.5%) of the 125 mycobacteria growing in BACTEC 12B cultures and to accelerate the identification of the three most current species of mycobacteria. This approach was tested four years ago with two different manual PCR methods for the detection of *M. tuberculosis* complex (6) and more recently with the manual Roche Amplicor PCR assay, but only for the detection of *M. tuberculosis* complex (14). With the development and availability of the commercial automated PCR system, this procedure has attracted further interest. The probes for *M. avium* and *M. intracellulare*, tested for the first time in this clinical application, appeared specific in this automatic PCR system. The same observation was previously reported with the same *M. avium* and *M. intracellulare* probes for the manual Amplicore MAI (Roche Molecular Systems) evaluated in positive blood cultures of 200 HIV-positive patients (10).

The addition of an internal control was an important improvement because false-negative PCR results due to amplification inhibition in positive specimens were revealed. However, the rate of amplification inhibition (19%) with positive BACTEC 12B cultures was surprisingly high compared to that of the procedure applied directly to respiratory sediments, where 4.7% of specimens were inhibited (2). The origin of these inhibitors remains unknown since about one-half of our inhibited specimens were collected from the respiratory tract. Furthermore, all samples were already diluted in the liquid culture medium, which should have decreased the inhibitors.

In a routine approach, we recommend performing the COBAS AMPLICOR assay on 1:10 diluted BACTEC 12B cultures with a GI of ≥100, when AFB are detectable after Ziehl-Neelsen staining. The advantage of this procedure is that contaminated or negative BACTEC cultures may be discarded and amplification inhibitors are reduced. This procedure would shorten the identification time by 3 or 4 days for *M. tuberculosis* complex, *M. avium*, or *M. intracellulare* with a correct identification in a unique amplification tube. Similar time savings were reported by Wobeser et al. (16), who analyzed 141 BACTEC 12B cultures and reported that PCR results for *M. tuberculosis* complex were available 4 days before the results of nucleic acid probe hybridization tests.

In conclusion, with the availability of new automated amplification systems, it is essential to evaluate these procedures under different clinical settings. An advantage of the COBAS AMPLICOR system is its capacity to perform multiple assays with a common platform and format (5). This system contributes to the general progress in automation in diagnostic laboratory medicine and to the flexibility in the selection of pathogens to be detected.

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